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(54) **Title:** SYSTEM AND METHOD FOR HIGH THROUGHPUT TISSUE DISRUPTION

(57) **Abstract:** An automated machine designed for high-throughput tissue disruption is disclosed. The machine includes a plurality of tissue disrupters, a moveable multi-well plate positioned below the tissue disrupters, and a moveable washing tray and dryer. The multi-well plate is configured to hold a plurality of separate tissue samples.

SYSTEM AND METHOD FOR HIGH THROUGHPUT TISSUE DISRUPTION

The invention disclosed herein relates to plant and animal tissue disruption and is advantageously applied to the extraction and/or purification of biological molecules or structures from the tissues.

Isolation of DNA, RNA and proteins from plant or animal tissue samples requires effective tissue disruption. While there are several methods of tissue disruption systems currently available, none of these systems provide for fast, high volume tissue disruption for use with high-throughput robotic purification systems, which is more and more required for agricultural and pharmaceutical studies.

One of the ways in which tissue disruption is accomplished is by employing a porcelain mortar and pestle. The use of the mortar and pestle dates back as early as 1550 B.C. for grinding and mixing ingredients together to make powders, poultices or ointments for medicine. While mortars and pestles are still commonly used in pharmaceutical preparations, they are ill-suited for high throughput tissue preparation. The use of a mortar and pestle requires manual grinding of tissue samples which can be labor intensive if many samples are to be tested. Only a single tissue sample can be prepared at a time with a mortar and pestle. Conical ground glass tissue grinders are also used for tissue disruption but have the same drawbacks as the mortar and pestle. These tissue grinders are essentially scaled down versions of the mortar and pestle and only one tissue sample can be prepared at a time.

Commercially available homogenizers and electric blenders have likewise been utilized to disrupt tissues for isolation of micro molecules. Homogenizers are machines that blend or emulsify a substance by forcing the substance through fine openings against a hard surface. Homogenizers overcome some of the limitations of mortar and pestle tissue preparation inasmuch as a variety of tissue samples can be disrupted and ground more quickly with a motorized homogenizer than if prepared manually. However, homogenizers are still only capable of preparing a single tissue sample at a time. While there are many types of laboratory homogenizers that are commercially available, none of the homogenizers are designed for high throughput tissue disruption.

Recently, commercially available bead mills and freezer mills have been employed for the disruption of multiple tissue samples. Examples of commercial bead mill suppliers include Industrial Milling Beads of Longmont, Colorado and Micro Grinding Systems of Little Rock, Arkansas. However, freezer mills is not well-suited for the multi-well setup that is required for most of the high throughput operation systems. Bead mills disrupt tissue ineffectively and often result in loss of tissue sample due to the non-specific binding of the samples to the beads. Freezer mills are similarly unsatisfactory inasmuch as they are extremely difficult to operate and require the addition of large quantities of liquid nitrogen.

In one aspect of the present invention, there is provided a method of parallel tissue disruption. Tissue disruption may be accomplished by placing tissue samples into sample wells and substantially simultaneously, inserting a corresponding number of tissue disrupters into the sample wells. The tissue samples are then disrupted.

In another aspect of the present invention, a high throughput system for tissue disruption includes a tissue disrupter head with an array of tissue disrupters attached to the disrupter head. Optionally, a washing and drying system are included in the high-throughput system.

The system may include a head with numerous tissue disrupters attached to the head. Preferably, the tissue disrupters have various disrupter tips attached. In one embodiment of the present invention, the disrupter tips are designed to grind tissue samples. The high throughput system may additionally include a multi-well sample plate positioned beneath the head of the disrupter. Advantageously, the multi-well sample plate includes numerous separate sample wells to contain numerous individual tissue samples. The disruption of the tissue samples may be accomplished by moving the head, the multi-well plate, or both so as to alter the relative positions of the head and the multi-well plate. Preferably, the relative position of the head and multi-well plate is alterable so that the disrupter tips are substantially simultaneously capable of being inserted into at least some of the separate sample wells. Optionally, the system additionally includes a moveable washing tray which is positionable beneath the head such that the disruption tips can be simultaneously inserted into the washing tray. The system may include a dryer configured to direct air flow at the plurality of tips.

In still another embodiment of the invention, there is provided an apparatus for simultaneously washing tissue disrupters. The washing apparatus includes a wash tray with a

fluid inlet coupled to a first chamber for receiving multiple grinding tips. Additionally, the washing apparatus has a fluid outlet for discharging fluid that is coupled to second chamber by a partition. The partition has a height which allows fluid to flow from one chamber, over the partition, and into the other chamber. Preferably, the partition creates fluid flow along a surface region of fluid from the first chamber.

A method of washing a plurality of tissue disrupters is likewise contemplated by the present invention. The method includes forming a reservoir of fluid with a flow therein, the flow being predominantly in a surface region of the fluid and immersing the tips of one or more tissue disrupters into the reservoir. The material released from the tips can flow away from the tips in the direction of the flow of the fluid. Optionally, the tissue disrupters are engaged while immersed in the reservoir to optimize the washing of the disrupters.

In another aspect of the invention, a method of separating compounds from tissue samples is provided. Numerous tissue samples may be placed in individual wells of a multi-well plate. The tissue samples are disrupted in parallel using an automated high throughput tissue disruption apparatus. Upon disruption of the tissue samples, the multi-well plate can be transferred to an automated liquid handling apparatus for the separation of compounds.

In yet another aspect of the invention, a method of parallel tissue disruption in preparation for high throughput compound separation is contemplated by the present invention. Tissue samples are provided into each of the individual wells of the multi-well plate and the tissue disrupters are inserted into each of a first set of individual wells of the multi-well plate. Advantageously, the tissue samples in the first set of individual wells are disrupted and the tissue disrupters are then removed. Upon removal of the tissue disrupters, the tissue disrupters may be washed. The tissue disrupters can then be inserted into a second set of individual wells of the multi-well plate and the tissue samples contained within the multi-well plates are then disrupted.

In another aspect of the invention, a tissue disruption apparatus is provided comprising a plurality of separate sample wells having tissue samples contained within the wells. The tissue disruption apparatus additionally includes a means for substantially simultaneously disrupting tissue contained in each of the separate sample wells.

Brief Description of the Drawings

Figure 1 is a flow chart representing the steps of obtaining purified chemicals from raw tissue samples.

Figure 2 is a perspective view of a high throughput tissue disruption apparatus.

Figure 3A is a detailed view of a tissue disruption apparatus with tissue disrupter tips inserted into tissue sample wells in a first position.

Figure 3B is a detailed view of a tissue disruption apparatus with the tissue disrupter tips inserted into tissue sample wells in a second position.

Figure 3C is a detailed view of a tissue disruption apparatus with the tissue disrupter tips inserted into tissue sample wells in a third position.

Figure 3D is a detailed view of a tissue disruption apparatus with the tissue disrupter tips inserted into tissue sample wells in a fourth position.

Figure 4 is a perspective view of a tissue disruption apparatus wherein the grinding tips are inserted into a washing system.

Figure 5 is a perspective view of a tissue disrupter.

Figure 5A is a perspective view of one embodiment of a grinding tip.

Figure 5B is a perspective view of an alternative embodiment of a grinding tip.

Figure 6 is a perspective view of the wash tray of a wash bath.

Figure 7 is an enlarged view of a grinding tip inserted into the top cover of a wash bath for drying.

Figure 8 is an exploded view of tissue disruption tips being dried.

Figure 9 is an enlarged view of a grinding tip inserted into the top cover of a wash bath for drying.

Embodiments of the invention will now be described with reference to the accompanying Figures, wherein like numerals refer to like elements throughout. The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner, simply because it is being utilized in conjunction with a detailed description of certain specific embodiments of the invention. Furthermore, embodiments of the invention may include several novel features, no single one of which is solely responsible for its desirable attributes or which is essential to practicing the inventions herein described.

In one aspect of the present invention, a method of parallel tissue disruption in preparation for chemical extraction is provided. Figure 1 is a block diagram of one high throughput method of isolating biological material from tissue samples. At block 10, tissue samples are prepared, for example by cutting or punching material from plant leaves or stems. The tissue samples may comprise any suitable organic composition for obtaining purified compounds or chemicals including plant samples, animal tissue samples, fish samples, avian samples, insect samples, fungal samples, and bacterial samples. In a preferred embodiment, the tissue samples are animal tissue samples. In a particularly preferred embodiment, the tissue samples are plant samples such as sections of leaves, stems, flowers, seeds, or roots. The tissue samples can be fresh or frozen. Preferably, the tissue samples are between 100 and 200 milligrams in weight.

At block 12, the tissue samples are placed in a multi-well plate. The multi-well plate can have a wide variety of configurations of receptacles/wells for holding a plurality of separate samples, any of which is suitable for use with the invention. Multi-well plates having rectangular arrays of wells are most commonly commercially available, but other configurations may also be used. Suitable multi-well plates are commercially available from a variety of suppliers including Matrix of Hudson, NH, BD Laboratories of Franklin Lakes, NJ, and Nunc of Rochester, NY. Each individual sample well of the multi-well plate can contain a tissue sample from the same tissue source. Alternatively, different wells of the multi-well plate can contain different tissue samples. The wells can have rounded bottoms. Advantageously, the bottoms of the multi-well plates are flat-bottomed.

Generally, the wells of the multi-well plates include fluid in which the tissue sample is suspended. The fluid may comprise any buffer, solvent, aqueous solution, gel, or other material. The fluid can be added before or after the tissue samples are placed into the multi-well plates. With approximately 100 mg samples and standard 96 well of 2 ml multi-well plates, about 0.4 to 0.5 ml of extraction buffer may advantageously be added to each well to avoid degradation of the biological molecules within the tissue samples. A wide variety of suitable extraction buffers are well known to those in the art and may be used in conjunction with the invention.

With reference to block 14, the multi-well plate containing the tissue samples may then be routed to an automated high-throughput tissue disrupter. In accordance with one aspect of

the invention, the high throughput tissue disrupter processes the tissue samples in a plurality of sample wells at substantially the same time. This increases the efficiency and reduces the cost of sample processing. Tissue disruption may be performed with grinders, sonicators, or other tissue disruption techniques. One embodiment of a suitable high throughput tissue disrupter which may be used is described in detail below. In this particular embodiment, the automated high-throughput tissue disrupter is comprised of a tissue disrupter head with numerous tissue grinding tips attached. In this embodiment, the operation of the high throughput tissue disrupter system may be automated via an electronic control unit as will be described below with reference to Figure 2.

Turning to block 16, the tissue samples can be routed to a commercially available liquid handling apparatus for the isolation and purification of various biological structures and molecules such as nuclei, organelles, membrane fragments, DNA, RNA, flavonoids, proteins, and the like after the tissue samples are disrupted. Suitable liquid handling apparatuses are well known by one of ordinary skill in the art, and are commercially available from, for example QIAGEN of Carlsbad, CA or ZYMARK of Boston, MA.

The disrupted tissue samples can be placed in an automated liquid handling apparatus for isolation and purification of DNA and RNA, gene expression profiling, molecular diagnostics, as well as for isolation and/or purification of chemical compounds or subcellular organelles. Disrupted tissue samples may also be provided to automated liquid handling apparatus for use with PCR technology, diagnostics, immunoassays, enzyme assays, metabolite assays, and forensics. Thus, placing tissue samples into a multi-well plate, routing the plate to an automated high throughput tissue disrupter, and then routing the disrupted samples to an automated liquid handling apparatus results in a highly efficient and cost effective tissue and chemical handling process and system, avoiding time consuming and expensive labor involved in conventional tissue disruption techniques.

Figure 2 illustrates one embodiment of an automated high-throughput tissue disruption apparatus 20. The high-throughput tissue disruption apparatus 20 comprises a head 22 having an array of tissue disrupters 24 attached thereto. The array of tissue disrupters 24 allows a plurality of tissue samples to be disrupted in parallel, thereby increasing the speed and efficiency of the tissue preparation process. The number of tissue disrupters 24 in the array can vary widely. In one embodiment adapted for use with 96 well multi-well plates, twenty-four

tissue disrupters are provided in the array. As illustrated in Figure 2, the tissue disrupter heads 24 may be connected to corresponding independent motors 26 via flexible drive shafts 28, only one of which is shown in Figure 2 for clarity of illustration. In this embodiment, the number of independent motors 26 which drive the tissue disrupters 24 corresponds to the number of tissue disrupters 24 in the high-throughput tissue disruption apparatus 20. This allows independent control of the speed and duration of disruption for each tissue disrupter if desired. However, in many applications, a single motor could be used which simultaneously drives all of the tissue disrupters.

The tissue disrupters 24 comprise a tissue disruption tip 30 which extends downward from the bottom surface of the head 22. The tip 30 may be configured to perform a wide variety of tissue disruption functions, including grinding, sonication, and homogenization. The disruption tip 30 is advantageously configured for insertion into standard size and shape wells of commercially available multi-well plates. The motor/disrupter/tip combination can be constructed in accordance with principles well known in the relevant art. In one embodiment, the motor 26 and drive shaft 28 are based on the motor model 395 and shaft model 225 from Dremel of Racine, WI. Preferably, however, the tip of this model is substituted with a smaller tip which is adapted for both insertion into the wells of commercially available multi-well plates and efficient disruption of tissues contained therein. These tips 30 are described in additional detail below with reference to Figures 5, 5A, and 5B.

The apparatus also advantageously includes a platform 32 positioned generally beneath the head 22 that supports a multi-well plate 36 thereon. As described above, any commercially available multi-well plate 36 is suitable for use in the present invention.

In the embodiment of Figure 2, an elevator arm 40 moves the tissue disrupters 24 relative to the multi-well sample plate 36. The head 22 of the apparatus is affixed to the elevator arm 40. The elevator arm 40 is configured to raise and lower the tissue disrupter tips 30 into the multi-well sample plate 36. In addition, the platform 32 on which the multi-well plate 36 is mounted is configured to be moveable so that the array of tips 30 is sequentially placed into all of the wells of the multi-well plate 36.

During operation, the high-throughput tissue disruption apparatus 20 employs this array of tissue disrupters 24 in combination with components which are moveable relative to one another in an automated fashion to perform the tissue disruption process in a fast and efficient

manner. By employing a plurality of positions, all of the samples housed in the individual wells of the multi-well plate 36 can be disrupted using considerably fewer tissue disrupters 24 than the number of individual wells. In a preferred embodiment, the tissue disruption apparatus 20 is designed to disrupt 96 tissue samples in a 96-well plate. As shown in Figure 2, this embodiment may have twenty-four tissue disrupters 24 and a four position motion configuration that advances the multi-well plate 36. However, it can be appreciated that when more or less tissue samples are disrupted, there may be more or less positions of the tissue disrupters. For example, if 384 tissue samples are to be disrupted, the tissue disruption apparatus may assume a 16 position motion configuration. Similarly, if 48 samples are being disrupted, the tissue disruption apparatus may operate in a two position configuration.

The movement of the elevator arm 40 and platform 32 may be controlled in a variety of ways. In one embodiment, the elevator 40 and platform 32 are mounted to precision air slides. The operation of air slides is well known by one of ordinary skill in the art. Preferably, the air slides include adjustable end stops and limit switches. The activation of the air slides is controlled through solenoid air valves. Optionally, airflow rate regulators are included on the slides to adjust the rate of movement. Because the operation of the apparatus 20 is based on a few specific relative configurations of disrupter tips 30 and multi-well sample plates 36 such that precise control of position between these specific positions is not required, air slides with end stops are advantageous in that they are relatively inexpensive components for accomplishing this function. However, it will be appreciated that a wide variety of options are available, including motor controlled lead screws, etc.

In some embodiments of the invention, the tissue disruption apparatus may include a wash bath 42. The wash bath 42 may also be mounted to a platform 44 which allows motion in the y-direction toward the head 22. The platform 44 may also comprise an elevator arm 46. The elevator arm 46 moves the wash bath 42 upward to immerse the tips 30 into a reservoir of water contained in the wash bath 42. Movement of the platform 44 and elevator arm 46 may also be controlled by precision air slides. The wash bath 42 is configured to receive the tissue grinding tips 30 of the tissue disrupters 24. After each disruption operation, the tips 30 are lifted to their maximum height directly over the multi-well sample plate 36. The wash bath 42 is moved into place horizontally and vertically under the tips 30. The tips 30 are inserted into

the wash bath 42 after each disruption cycle as described further below with reference to Figure 4.

The motors 26 and air slides may be controlled by an electronic control unit 50. The control unit 50 advantageously includes a digital microcontroller having control outputs. The control outputs are coupled to compressed air solenoid valves and motor controllers to control the system operation.

Figures 3A-3D illustrate the four positions of the tissue disrupter when as many as 96 tissue samples are being disrupted. Figure 3A illustrates the first position of the tissue disrupters 24 of tissue disruption apparatus 20. Tissue is disrupted by inserting the grinding tips 30 of the tissue disrupters 24 into a first set of the individual wells of a multi-well plate 36. The controller 50 activates the disruption activity of the tissue disrupters 24, causing the grinding tips 30 to move vertically into and out of the wells of the multi-well plate 36. The motion of the tissue disrupters 24 causes the tissue samples to be disrupted. The tissue disrupters 24 are then raised to their maximum height. Preferably, a wash bath is moved into position under the tissue disrupter 24 (not shown). The tissue disrupters 24 are inserted into the wash bath and rinsed. Optionally, the tissue disrupters 24, particularly the grinding tips 30, are dried with an air dryer formed as part of the wash tray as will be described with reference to Figure 8. The tissue disrupters 24 are then inserted into a second set of individual wells of the multi-well plate 36 as is illustrated in Figure 3B. Figure 3B depicts the second position of the tissue disruption apparatus 20. The tissue grinding tips 30 are inserted into the second set of individual wells of the multi-well plate 36 and the controller 50 activates the disruption activity. Upon disruption of the tissues, the grinding tips 30 are again washed and advantageously dried. Then, the tissue disrupters 24 are moved into a third position and inserted into another set of individual wells of the multi-well plate 36 as illustrated in Figure 3C. Figure 3D depicts the fourth position of the tissue disrupters 24 in the fourth set of individual wells of the multi-well plate 36. Thus, by positioning the multi-well plate 36 in a series of four orientations relative to the array of disrupters, 24 tips are used to disrupt 96 tissue samples.

Figure 4 is a perspective view of a tissue disruption apparatus 20 with the grinding tips 30 of the tissue disrupters 24 inserted into the wash bath 42. The wash bath 42 can include a top cover 48 configured with a plurality of tissue disrupter receptacles 52. Preferably, the

number of tissue disrupter receptacles 52 corresponds with the number of tissue disrupters 24 to be washed. The wash bath 42 further includes a wash tray 54. Encased within the wash tray 54 is a washing solution. As used herein, a washing solution may include water, any cleaning solution, solvent, detergent, or aqueous solution capable of causing tissue debris to be removed from the surface of a tissue disrupter, sonicator, probe, or other instrument. The wash tray 54 advantageously incorporates a continuously flowing fluid bath. A detailed description of the wash tray 54 is presented below with reference to Figure 6.

After each grinding operation, the tissue disrupters 24 are lifted to their maximum height directly over the wells of the multi-well plate 36. The wash bath 42 is attached to an elevator arm 46. The wash bath 42 may also be mounted to a platform 44. The platform 44 extends to horizontally position wash bath 42 under the grinding tips 30 of the tissue disrupters 24. The elevator arm 46 raises the wash bath 42 until the grinding tips 30 of the tissue disrupters 24 are inserted into the tissue disrupter receptacles 52 of the wash bath 42. Advantageously, the grinding tips 30 are activated while submerged in the wash bath 42 to improve the effectiveness of the washing. While the grinding tips 30 are in the wash bath 42, the multi-well plate 36 is indexed to the next operating position by the electronic controller 50. Performing this wash procedure between each disruption cycle helps to prevent cross-contamination between individual samples within the multi-well plate 36.

Figure 5 illustrates one embodiment of a tissue disrupter 24 of the present invention. The tissue disrupter 24 is comprised of a drive head 56 attached to a flexible drive shaft such as the drive shaft 28 illustrated in Figure 1. The flexible drive shaft attaches to a drive motor, again as illustrated in Figure 1. Additionally, the tissue disrupter 24 includes a grinding sleeve 58. The diameter of the lower portion 59 of the grinding sleeve 58 is configured to fit within a standard multi-well plate. Generally, the lower portion of the grinding sleeve 59 will have a has a diameter of between about 2.0 to 7.0 mm, depending upon the size of the individual sample wells within a multi-well plate being used. In one embodiment, the grinding sleeve 58 has a diameter of approximately 6.6 mm when the multi-well plate is a standard 96-well plate. In an embodiment suitable for multi-well sample plates containing 384 individual sample wells, the sleeve 59 may have a diameter of approximately 2.5 mm.

Disposed within the drive head 56 and grinding sleeve 58 is a grinding tip 30. The grinding tip 30 is attached to the drive head 56 in a collate arrangement. Advantageously, the

grinding tip 30 is removable. The grinding tip may be made of any suitable material including a metal or metal alloy such as 316 stainless steel, a ceramic material, or even plastic.

Various grinding tips have utility in the present invention. Preferably, the grinding tips are removable. The grinding tip 30 can be changed for disrupting different types of tissue. For example, softer tissue may disrupted using a grinding tip such as the one illustrated in Figure 5A. With reference to Figure 5A, there is a shank 60 at the distal end 62 of the grinding tip 30. The shank 60 is chamfered at a 45 degree angle.

At the proximal end 64 of the grinding tip 30, there is provided a grinding blade 66. It should be appreciated by one of ordinary skill in the art that the blade 66 may have a wide variety of configurations, two of which are illustrated in Figures 5A and 5B. The blade of Figure 5A, for example, may have a flat or curved proximal edge. The blade configuration of Figure 5A is particularly advantageous for grinding soft tissue samples such as leaves, soft organ tissues, etc. Figure 5B illustrates an alternative embodiment of a grinding tip 30. In this embodiment, the blade 70 is comprised of a fork-like, pronged end 72 with a concavity created between the two forks of the pronged end 72. The blade 70 is twisted about the center of the grinding tip 30 by 45 degrees over the length of the blade. The leading edges of the twist narrow to form sharp, flat edge forks. Alternatively, the blade of the grinding tip 30 may have a plurality of flutes or a spiral cutting pattern like an endmill for grinding harder tissue samples such as dense plant material, namely roots, seeds, bark, and stems and dense animal tissue samples such as bone.

Figure 6 is a perspective view of a wash tray 54 of a wash bath apparatus. The wash tray 54 includes at least one fluid inlet 72 and at least one fluid outlet 74 for discharging fluid. The wash tray 54 further comprises at least one chamber 76 coupled to the fluid inlet 72. A second chamber 78 is coupled to the fluid outlet 74 and is separated from the first chamber 76 by a partition 80. The partition 80 is configured to have a height which allows fluid to flow from the first chamber 76, over the partition 80, and into the second chamber 78. Fluid flow along a surface region of fluid in the first chamber 76 is thus created. When the grinding tips are immersed from above into the first chamber 76 in a wash bath of this design, the washing solution flows around and past the tips, along the surface, over the partition 80, and into the second chamber 78, taking debris and contaminants away from the immersed tips. As shown in this Figure, the washing tray may additionally include a third chamber 82 coupled to a fluid

outlet 84. A second partition 86 allows fluid to flow from the first chamber 76, over the partition 86, and into the third chamber 82. In such a configuration, the first chamber 76 is located between the second chamber 78 and third chamber 82.

The flow of fluid and drainage of fluid in the wash tray 54 is controlled by the controller 50. Preferably, fluid flow and drainage operate only during the washing process to minimize the amount of fluid being utilized. However, in an alternative embodiment, the fluid flow is constant. A fluid source reservoir is connected to the fluid inlet 72. Additionally, a fluid discard reservoir is coupled to the fluid outlet 74. A pump in communication with the controller 50 is attached to the fluid source reservoir and fluid discard reservoir. The entire wash tray 54 may be drained and refilled between wash times while the tissue disrupters 24 are grinding the tissue samples. Alternatively, the wash tray 54 may be completely drained and refilled after all of the tissue samples in a multi-well plate 36 are disrupted and prior to introducing a new multi-well plate containing new tissue samples into the tissue disrupter apparatus 20.

The wash tray 54 may further comprise a wash tray cover 48, shown in Figures 2 and 4. The wash tray cover would include a plurality of tissue disrupter receptacles or openings for receiving the grinding tips of the tissue disrupters.

Turning now to Figure 7, a system for drying the grinding tips 30 is preferably included in the tissue disruption apparatus 20. In one embodiment, the top cover of the wash tray includes a compressed air tip dryer. The dryer may be formed in the top cover of the wash tray by forming the top cover with a hollow central region that is coupled to a source of compressed air. A wash bath cover incorporating this feature is illustrated in Figures 7, 8, and 9.

Figure 7 is an enlarged side view of one tissue disrupter receptacle 52 on the top cover 48 of the wash tray. During the wash cycle, a grinding tip 30 is inserted into and through the receptacle and into the chamber of the wash tray 54 containing the wash solution. In this embodiment, the top cover 48 is formed by a top plate 92 and a bottom plate 94 defining a hollow region 96 in between. Each receptacle 52 in the top cover 48 which receives a tip 30 includes a downwardly extending tube 98 which is integral to the top plate 92 and which extends downward until it almost contacts the bottom plate 94. The bottom plate 94 is sloped downward to form an angled portion 104 of the bottom plate 94. The bottom portion 100 of

the tube 98 likewise has angled sides to create a substantially close fit between the tube and an opening in the bottom plate 94 through which the tip 30 extends when inserted into the wash tray 54. This produces a circumferentially extending gap 102 between the tube 98 and the bottom plate 94 which is coupled to the hollow interior region 96 of the top cover 48. The interior region 96 may be connected to an air source which is also in electronic communication with the controller (not shown). Advantageously, the air source directs pressurized air through the gap 102 between the bottom 100 of the tube 98 and the opening in the bottom plate 94 so as to direct the air downward and at the inserted tips 30. Alternatively, the central region 96 may be coupled to a vacuum pump that pulls air upward over the grinding tips 30 and through the gap 102 between the bottom of the tube 98 and the opening in the bottom plate 94. In either embodiment, the air flow operates to dry the disruption tips.

Figure 8 illustrates an exploded view of disruption tips extending through the tubes 98 and the opening in the bottom plate 94. As the wash tray 54 is lowered slowly following the wash cycle by the protraction of the elevator arm and the tips are removed from the reservoir through the tube 98, the air flow removes the fluid from the grinding tips 30.

Figure 9 illustrates an alternative embodiment of the top cover of the wash bath. Figure 9 is an enlarged side view of one tissue disrupter receptacle 52 on the top cover 48 of the wash tray. As was described with reference to Figure 7, a grinding tip 30 is inserted into and through the receptacle and into the wash tray containing the wash solution during the wash cycle. The bottom plate 94 includes a raised, upwardly extending portion 104 with angled sides. Advantageously, a downwardly extending tube 98 configured to receive the grinding tip 30 is affixed to the top plate 92. The lower portion 100 of the tube 98 likewise has angled sides to create a substantially close fit between the tube 98 and the bottom plate 94. This produces a circumferentially extending gap 102 between the tube 98 and the bottom plate 94 which is coupled to the hollow interior region 96 of the top cover 48. The interior region 96 may be connected to an air source as was described with reference to Figure 7. Advantageously, the air source is pressurized air directed through the gap 102 between the lower portion 100 of the tube 98 and the bottom plate 94 so as to direct the air downward and at the inserted tips 30. Alternatively, a vacuum directs negative air flow at the plurality of grinding tips 30 to dry the grinding tips 30.

In accordance with the foregoing, certain embodiments of the invention provide an automated system for tissue disruption in a high-throughput platform, which will accelerate research progress for molecular cloning, expression profiling, compound and drug screening, molecular diagnostics in agricultural and pharmacological researches. It will be appreciated, however, that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should be noted that the use of particular terminology when describing certain features or aspects of the invention should not be taken to imply that the terminology is being re-defined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. The scope of the invention should therefore be construed in accordance with the appended claims and any equivalents thereof.

What is claimed is:

1. A system for high throughput tissue disruption comprising a head having a plurality of tissue disrupters attached thereto.

2. The system of Claim 1 wherein the plurality of tissue disrupters comprise disruption tips configured to fit into a well of a multi-well plate.

3. The system of Claim 2, wherein the tips are arranged in a substantially rectangular array such that each of the tips can be simultaneously inserted into a substantially rectangular array of wells.

4. A system for high throughput tissue disruption according to any one of Claims 1 to 3 comprising:

a head having a plurality of tissue disrupters attached thereto and a multi-well plate comprising a plurality of separate sample wells to contain a respective plurality of tissue samples, wherein one or both of said head and said multi-well plate is moveable so as to alter the relative position of said head and said multi-well plate;

a plurality of disrupter tips attached to the tissue disrupters, wherein the plurality of tips are configured to grind tissue samples, and wherein the relative position of said head and said multi-well plate is alterable so that said plurality of disrupter tips are substantially simultaneously insertable into at least some of said plurality of separate sample wells.

5. The system of any one of Claims 1 to 4, wherein said system comprises an elevator configured to raise and lower the tissue disrupters to removably insert the tissue disrupters into the sample wells.

6. The system of any one of Claims 1 to 5, wherein said head is stationary.

7. The system of any one of Claims 1 to 6, wherein said multi-well plate is mounted on a moveable platform.

8. The system of any one of Claims 1 to 6, further comprising:

a platform supporting the multi-well plate; and

one or more compressed air pistons coupled to the platform for moving the multi-well plate.

9. The system of any one of Claims 1 to 6, further comprising:

a platform supporting the multi-well plate; and

one or more lead screws coupled to the platform for moving the multi-well plate.

10. The system of any one of Claims 1 to 9, wherein said system comprises an elevator configured to raise and lower the tissue disrupters to removably insert the tissue disrupters into the sample wells.

11. The system of Claim 10, further comprising one or more compressed air pistons coupled to the elevator for raising and lowering the tissue disrupters.

12. The system of any one of Claims 1 to 11, additionally comprising a moveable washing tray which is positionable beneath the head whereby the plurality of tips can be simultaneously inserted into the washing tray.

13. The system of Claim 12, additionally comprising a dryer wherein the dryer is configured to direct air flow at the plurality of tips.

14. The system of any one of Claims 1 to 13, further comprising a programmable logic circuit with control outputs coupled to the tissue disrupters and to the one or more compressed air pistons to control system operation.

15. An apparatus for tissue disruption which is designed such that it can be used in a system for high throughput tissue disruption comprising one or more of the elements provided in any one of claims 1 to 14.

16. An apparatus according to claim 15 comprising:
a plurality of separate sample wells having tissue samples contained therein; and
means for substantially simultaneously disrupting tissue contained in each of the plurality of separate sample wells.

17. A method of parallel tissue disruption comprising:
placing tissue samples into a plurality of sample wells;
substantially simultaneously inserting a plurality of corresponding tissue disrupters into the plurality of sample wells;
substantially simultaneously disrupting the tissue samples.

18. The method of claim 17, wherin the tissue samples and fluid are placed into a plurality of sample wells.

19. The method of any one of Claims 17 or 18, wherein the sample wells are the wells of a multi-well plate.

20. The method of Claim 19, additionally comprising the step of transferring the multi-well plate to an automated liquid handling apparatus.

21. A method of parallel tissue disruption in preparation for high throughput compound separation, the method comprising:

providing a tissue sample into each of a first plurality of separate sample wells and a second plurality of separate sample wells;

inserting one of a plurality of tissue disrupters into each of the first plurality of separate sample wells;

disrupting the tissue sample in each of the first plurality of separate sample wells;

removing the plurality of tissue disrupters from the first plurality of sample wells;

washing the plurality of tissue disrupters;

inserting one of the plurality of tissue disrupters into each one of the second plurality of separate sample wells; and

disrupting the tissue sample in each of the second plurality of separate sample wells.

22. The method of Claim 21, wherein the first plurality of separate samples wells are formed in a first multi-well plate, and wherein the second plurality of separate sample wells are formed in a second, different multi-well plate.

23. The method of Claim 21, wherein the first plurality of separate sample wells and the second plurality of separate sample wells are formed in a single multi-well plate.

24. The method of any one of Claims 17 to 23, wherein the multi-well plate is selected from a group consisting of 6-well plates, 12-well plates, 24-well plates, 48-well plates, 96-well plates, and 384-well plates.

25. The method of Claim 24, wherein the multi-well plate is a 96-well plate.

26. The method of any one of Claims 17 to 25, additionally comprising drying the plurality of tissue disrupters after washing the plurality of tissue disrupters.

27. A method according to any one of claims 1 to 26 comprising the additional step of separating compounds from tissue samples comprising:

placing a plurality of tissue samples in a multi well plate;

disrupting the tissue samples in parallel using an automated high throughput tissue disruption apparatus; and

transferring the multi-well plate to an automated liquid handling apparatus.

28. The method of Claim 27, further comprising separating the compounds from tissue samples.

29. In a system for high throughput tissue disruption, a method of substantially simultaneously washing a plurality of tissue disrupters comprising:

forming a reservoir of fluid having a flow therein, the flow being predominantly in a surface region of the fluid;

substantially simultaneously immersing tips of the one or more tissue disrupters into the reservoir, whereby material released from the tips flows away from the tips in the direction of the flow.

30. The method of Claim 29, additionally comprising operating the one or more tissue disrupters while the tips are immersed in the reservoir.

31. An system for simultaneously washing a plurality of tissue disrupters comprising:

a wash tray, the wash tray comprising:

at least one fluid inlet;

at least one fluid outlet for discharging fluid;

a first chamber configured for simultaneously receiving a plurality of tissue disrupters; wherein the first chamber is coupled to the fluid inlet;

at least a second additional chamber coupled to the fluid outlet and separated from the first chamber by a partition having a height which allows fluid to flow from the first chamber, over the partition, and into the second additional chamber, whereby fluid flow along a surface region of fluid in the first chamber is created such that when said plurality of tissue disrupters are immersed in said first chamber, surface fluid flow directs contaminants generally away from said tissue disrupters and into said second additional chamber.

32. The washing system of Claim 31, wherein the tray comprises a third additional chamber coupled to one of at least one of said fluid outlets and separated from the first chamber by a partition having a height which allows fluid to flow from the first chamber, over the partition, and into the third additional chamber.

33. The washing system of Claim 32, wherein the first chamber is located between the second chamber and the third chamber.

34. The washing system of any one of Claims 31 to 33, additionally comprising a wash tray cover, wherein the wash tray cover includes a plurality of openings for receiving tissue disrupter tips therethrough.

35. The washing system of Claim 34, wherein the cover comprises an inlet for compressed air and wherein at least some of the plurality of openings comprises an associated outlet for compressed air, whereby tissue disrupter tips are dried with compressed air as they are extracted from the openings after washing.

36. A method of making a tissue disruption apparatus comprising:
forming an array of tissue disrupters;
positioning a plate holder beneath the array.

37. The method of Claim 36, wherein forming the array of tissue disrupters comprises coupling a plurality of separate tissue disrupters to a common head.

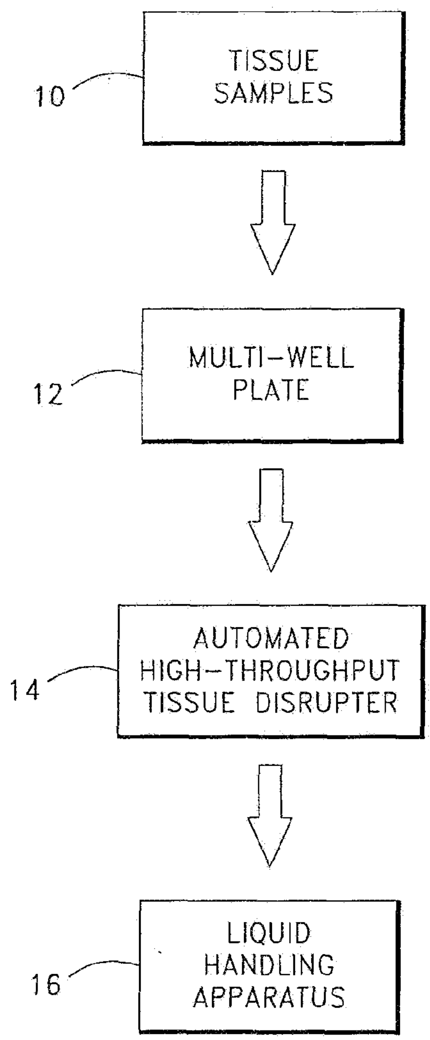
TISSUE SAMPLES
10
MULTI-WELL PLATE
12
AUTOMATED HIGH-THROUGHPUT TISSUE DISRUPTER
14
LIQUID HANDLING APPARATUS
16

Fig. 1

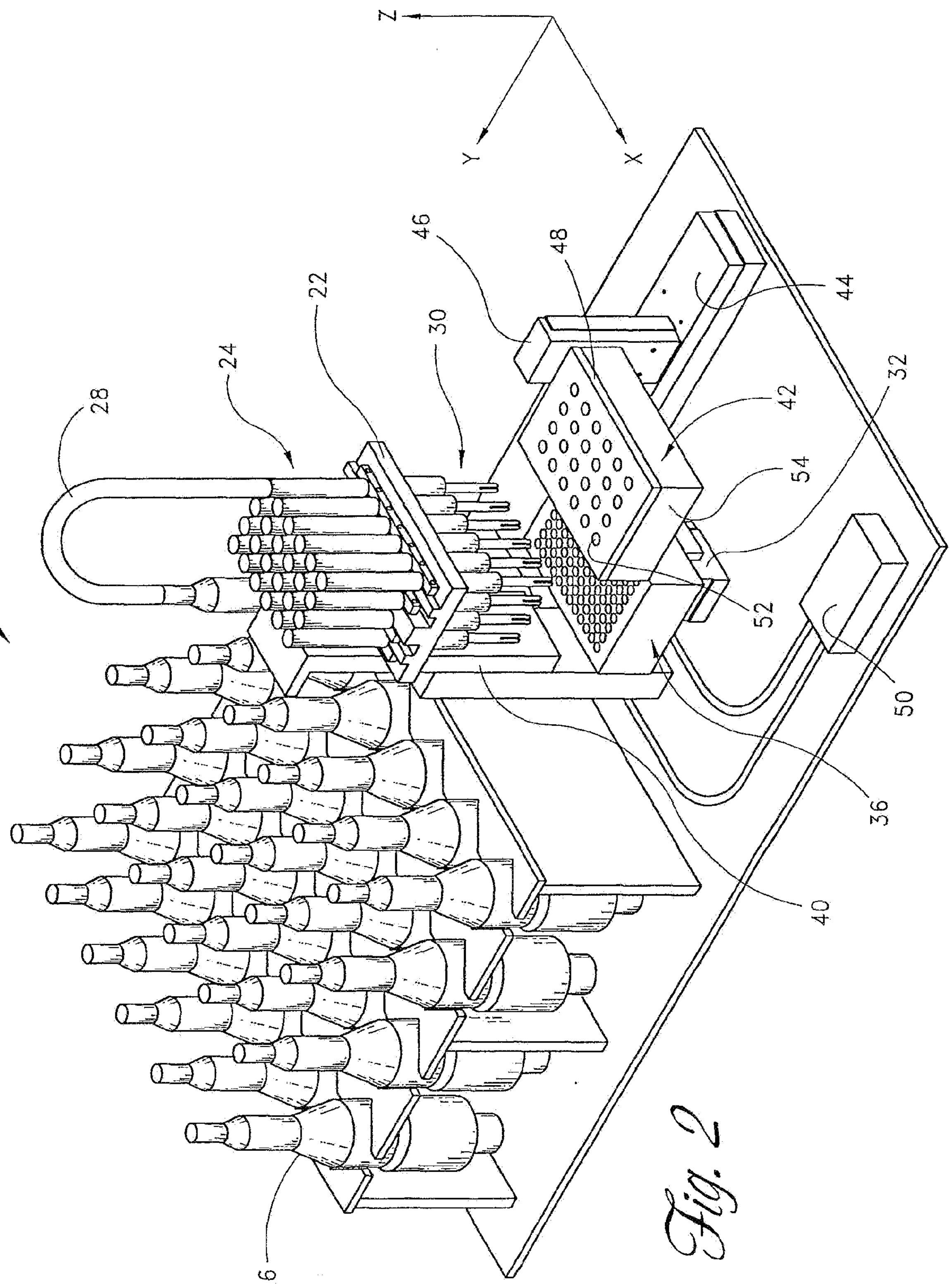

Fig. 2

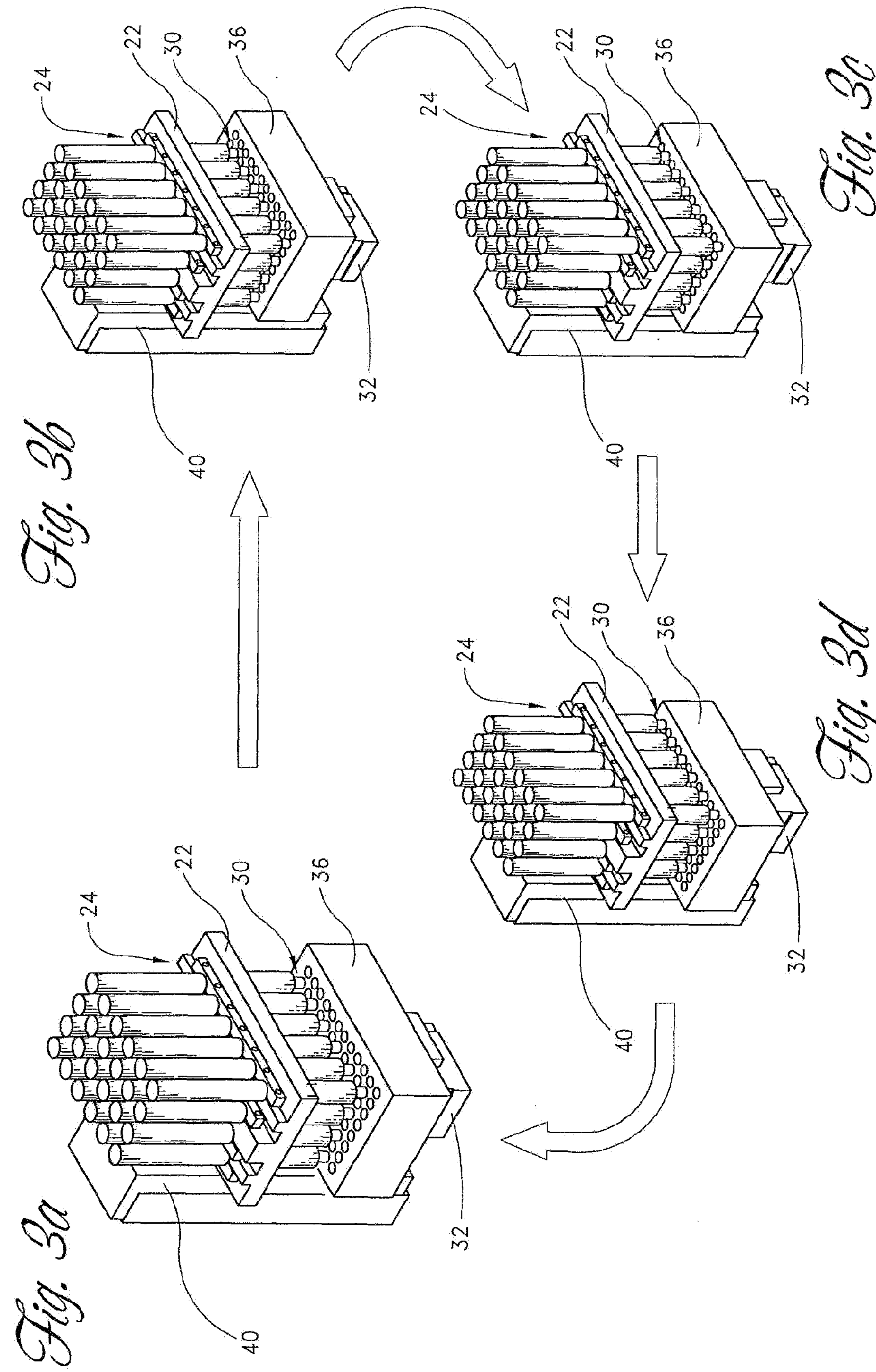

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3d

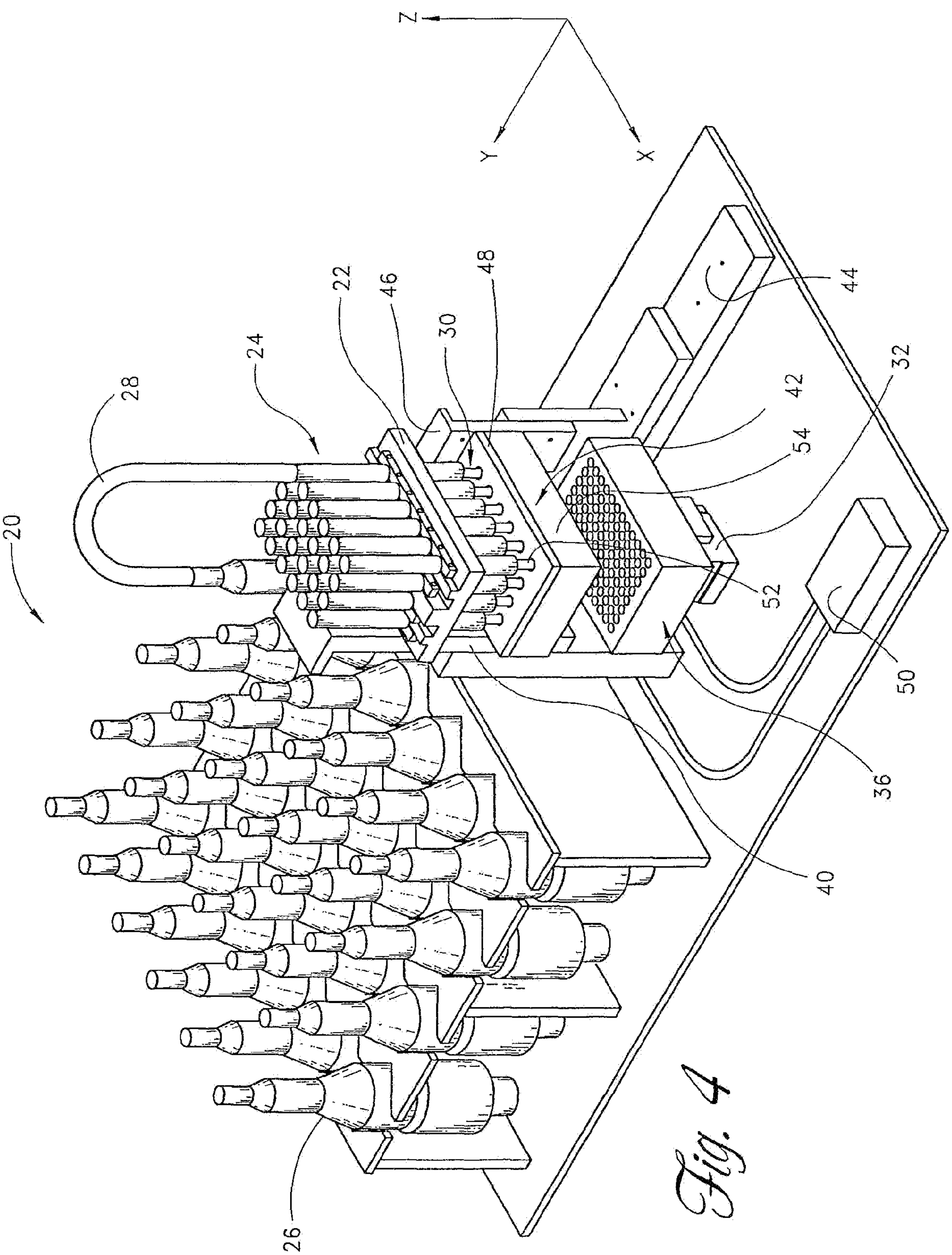

Fig. 4

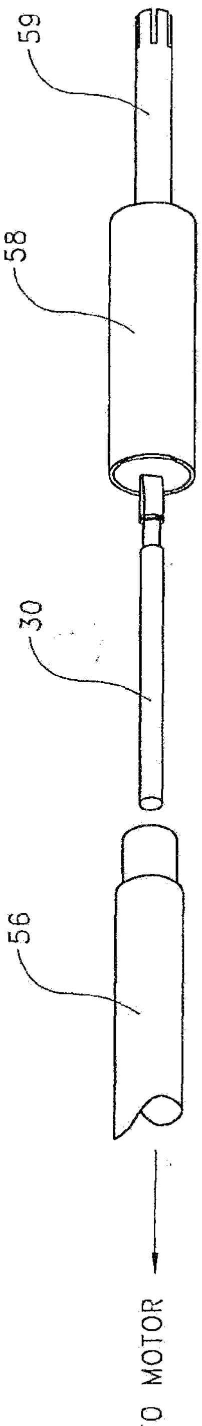

Fig. 5

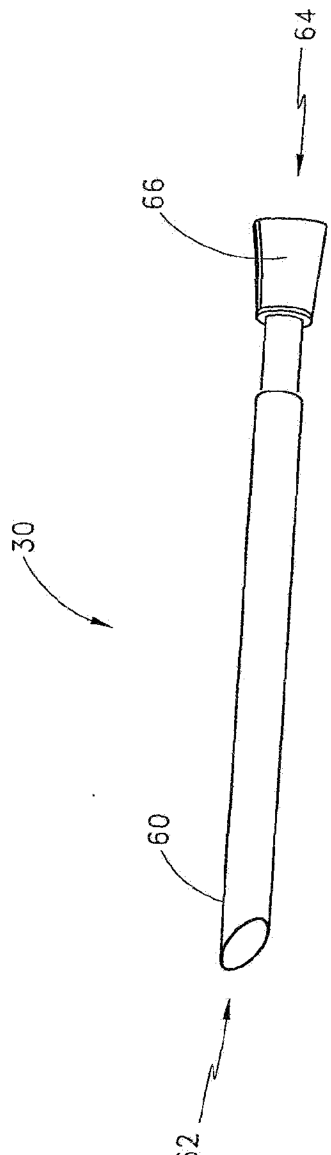

Fig. 5a

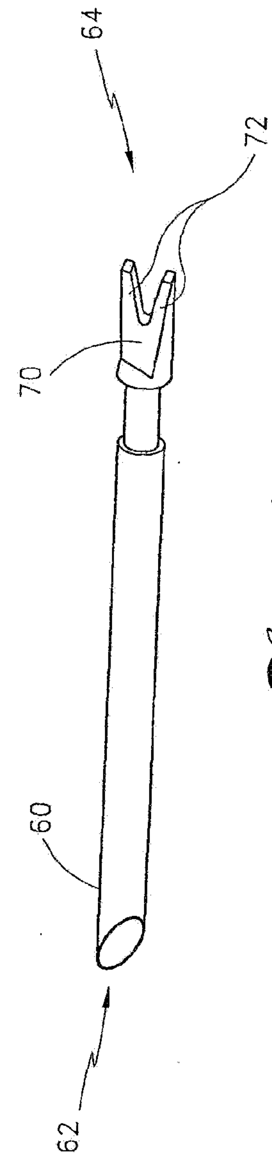

Fig. 5b

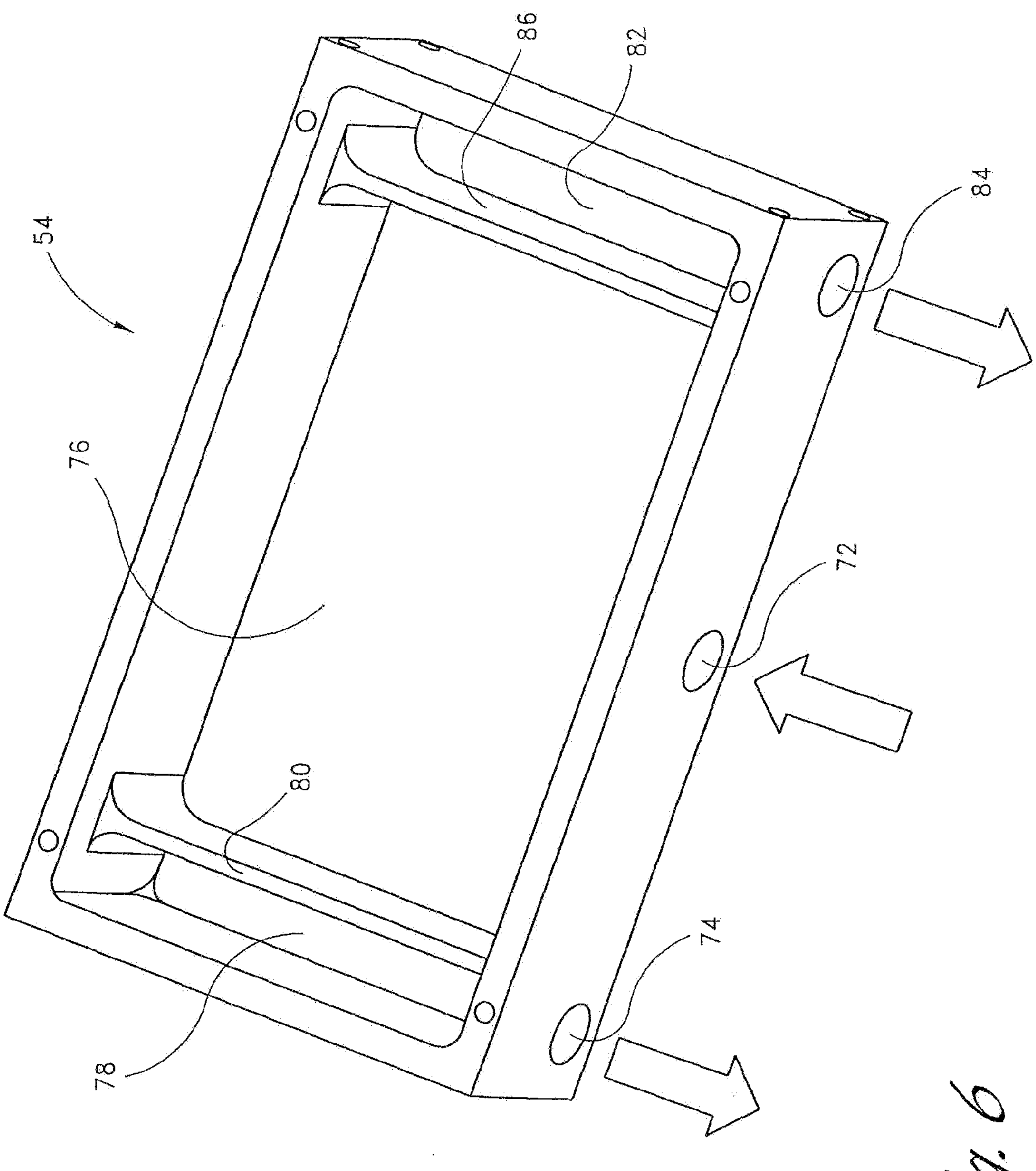

Fig. 6

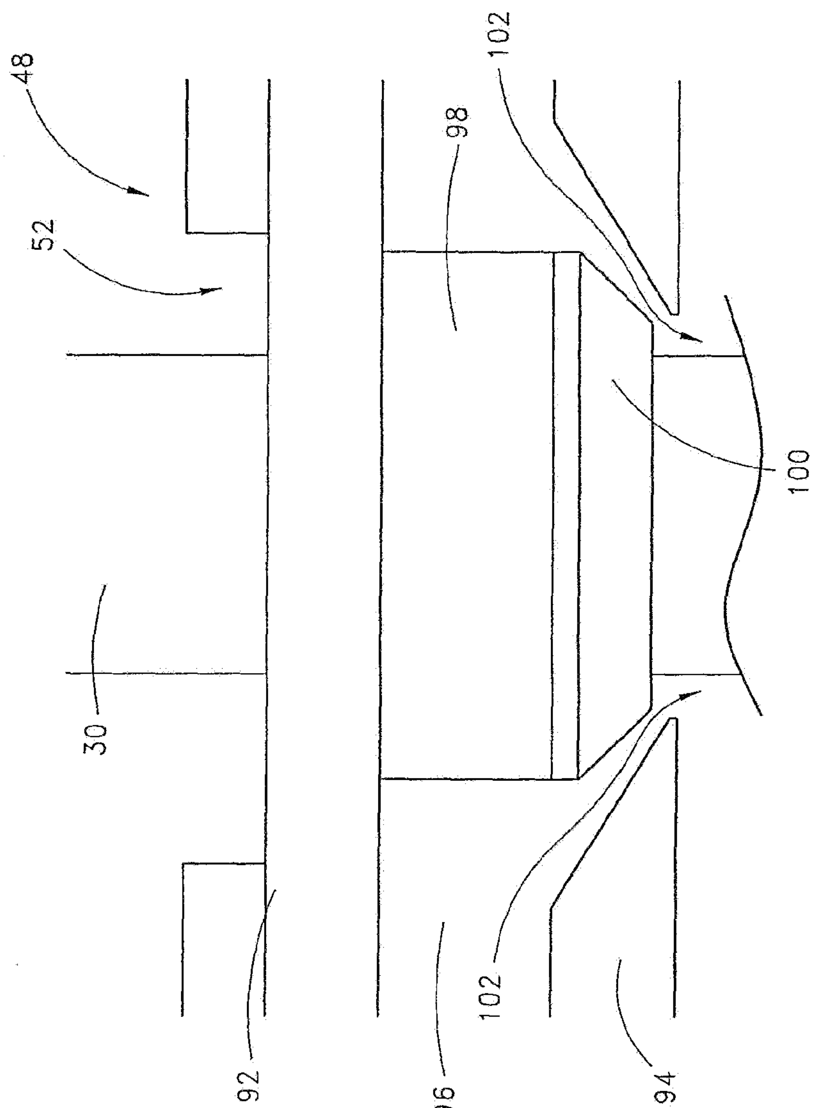

Fig. 7

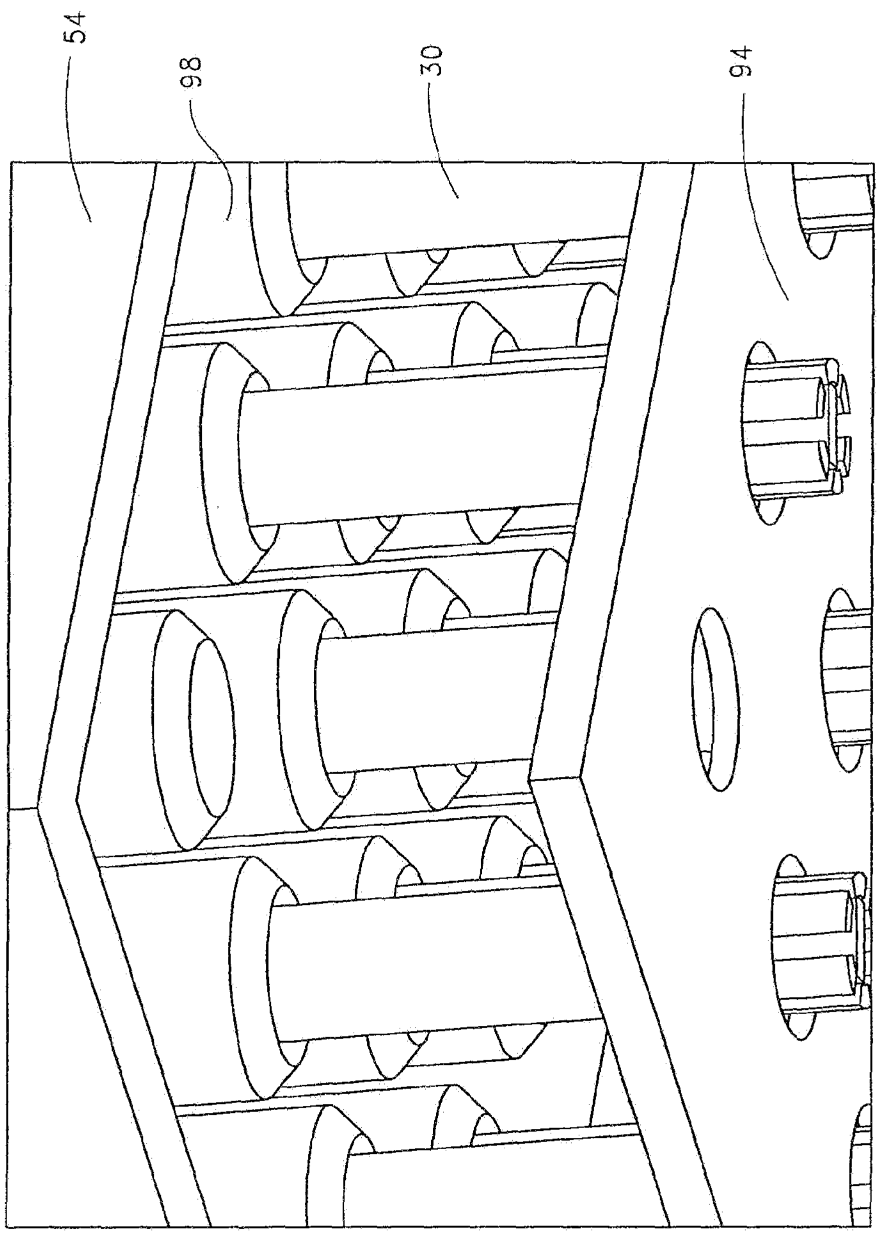

Fig. 8

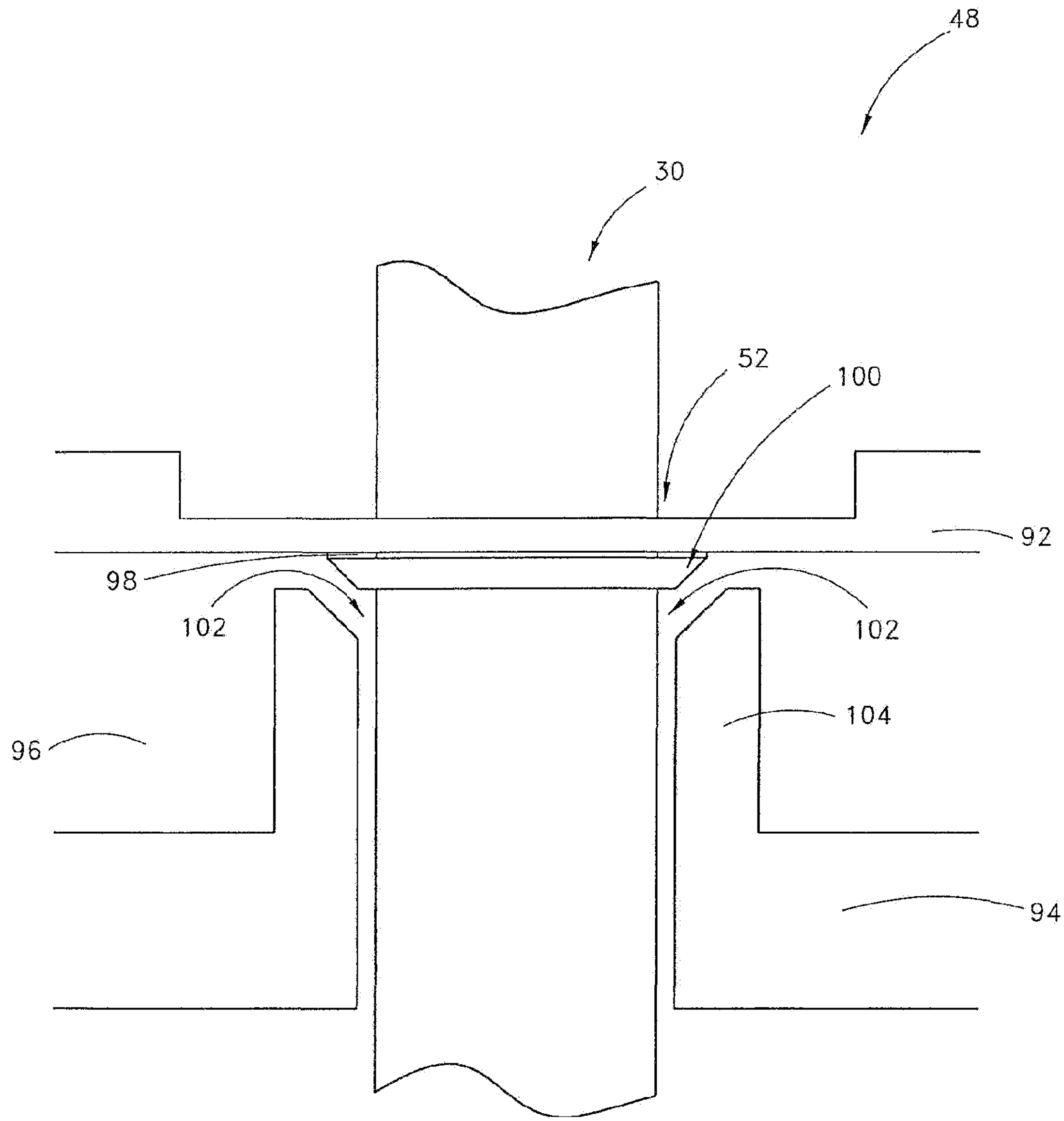

Fig. 9

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(72) Inventors; and
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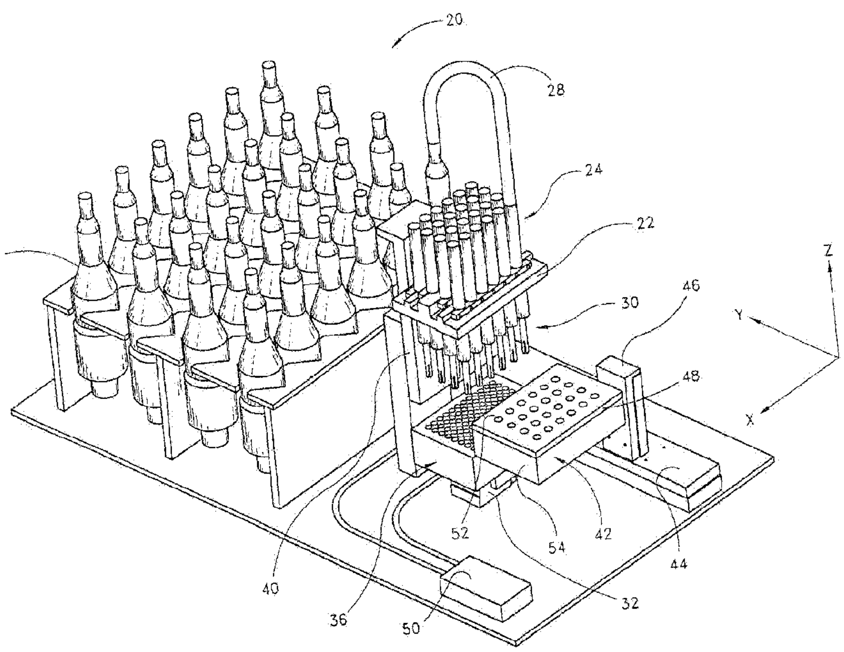
(54) Title: SYSTEM AND METHOD FOR HIGH THROUGHPUT TISSUE DISRUPTION



(57) Abstract: An automated machine designed for high-throughput tissue disruption is disclosed. The machine includes a plurality of tissue disrupters, a moveable multi-well plate positioned below the tissue disrupters, and a moveable washing tray and dryer. The multi-well plate is configured to hold a plurality of separate tissue samples.

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IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

Int al Application No
PCT/EP 01/06429

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N1/28 B01F7/16 C12M3/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 299 06 077 U (SAATEN UNION RESISTENZLABOR GM) 12 August 1999 (1999-08-12) the whole document	1-6,10, 15-20, 27,28, 36,37
A		7-9, 11-14, 21-26
P,X	DE 199 15 262 A (SAATEN UNION RESISTENZLABOR GM) 5 October 2000 (2000-10-05) abstract; figures 1,2 column 1, line 44 -column 2, line 45	1-6,10, 15-20, 27,28, 36,37
A		7-9, 11-14, 21-26

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 May 2002	03.09.02

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Runser, C

Form PCT/ISA/210 (second sheet) (July 1992)

Int al Application No
PCT/EP 01/06429

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 58637 A (KLEFFNER BERNHARD ;HAHN THOMAS (DE); RUF HANS (DE); FRAUNHOFER GES) 18 November 1999 (1999-11-18) abstract; figure 3 page 10 -page 12 ---	1,15,16, 36,37
A	WO 98 20164 A (CORNELL RES FOUNDATION INC) 14 May 1998 (1998-05-14) abstract; figures 1-6 page 3, line 10 -page 4, line 21 page 5, line 10 -page 7, line 10 -----	1-28,36, 37

INTERNATIONAL SEARCH REPORT

ıtional application No.
PCT/EP 01/06429

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-28, 36-37

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28,36-37

   System and method for tissue disruption

2. Claims: 29-35

   Washing system and method

INTERNATIONAL SEARCH REPORT

ımation on patent family members

Int al Application No
PCT/EP 01/06429

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
DE 29906077	U	12-08-1999	DE	29906077 U1	12-08-1999
DE 19915262	A	05-10-2000	DE	19915262 A1	05-10-2000
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			WO	9958637 A2	18-11-1999
			EP	1075507 A2	14-02-2001
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			WO	9820164 A1	14-05-1998

Form PCT/ISA/210 (patent family annex) (July 1992)